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# RECOMBINATION MECHANISMS IN BACTERIA

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ONE FIGURE

Several mechanisms of genetic recombination have been described or inferred in bacteria. Genetic recombination is taken to include any biological mechanism for the reassortment within one cell lineage of determinants from distinct sources. The present classification (table 1) is based on the scope of the unit of exchange. Most of its categories are exemplified among the bacteria. Cytoplasmic exchange, however, is not yet documented but may be suspected as a corollary of possible "disinfections" (Van Lanen *et al.*, '52; McIlroy *et al.*, '48; Bunting *et al.*, '51).

## GENERAL FEATURES OF RECOMBINATION MECHANISMS

### *Heterokaryosis*

Heterokaryosis is best known among the filamentous fungi for it consists of the coexistence of genetically different nuclei within a single cell or cytoplasmic field (Pontecorvo, '46). The persistent integrity of the constituent nuclei distinguishes heterokaryosis from sex; indeed, genetic exchange (karyogamy) may sometimes intervene without the overt paraphernalia of the sexual stage (Pontecorvo, '53; Papazian, '54). Heterokaryosis may be initiated by mutation within a coenocyte, by deferred nuclear separation after meiosis, or by anastomosis of cells, hyphae, or spores. The first two

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TABLE 1

*Mechanisms of genetic recombination*

| DESIGNATION | UNIT TRANSFERRED | AGENCY | FATE | EXAMPLES IN BACTERIA OR OTHER MICROBES |
|---|---|---|---|---|
| (1) Sex (karyogamy) | Intact nucleus | Union of cells, hyphae, or more or less specialized gametes or gametangia | Hybrid zygote nucleus may be followed by chromosome segregation, elimination, or crossing over. | Lederberg and Tatum, '53 |
| (2) Heterokaryosis | Intact nucleus | (Same as above) | Intact nuclei in common cytoplasmic field. May be terminated by (1) or by nuclear segregation. | *Streptomyces?*<br>(See Pontecorvo, '46) |
| (3) ? | Intact chromosome | ? | ? | Hypothetical (See Muller and Pontecorvo, '40) |
| (4) Genetic transduction | "Chromosome" fragment | Chemical preparations of DNA<br>Bacteriophage | Integration into genotype | Austrian, '52; Zinder and Lederberg, '52 |
| (5) Lysogenic conversion | Phage nucleus (prophage) [May be special case of (4)] | Infective phage | Incorporation unless doomed by bacteriolysis (Bacteriolysis may occur sporadically in later generations.) | Lwoff, '53 |
| (6) Cytoplasmic transfer | Plasmids (viruses, symbionts, plasmagenes, or other extranuclear hereditary factors) | Plasmogamy [usually associated with (1) or (2)]<br>Intercellular infection | Hereditary symbiosis | Hypothetical for bacteria (See Ephrussi, '53; J. Lederberg, '52; Sonneborn, '50) |

modes of heterokaryosis are only incidental to recombination, but cannot be ignored as transient phenomena in customarily multinucleate bacteria (Lederberg, '49a; Witkin, '51). Persistent heterokaryons could be expected only in filiform bacteria such as the actinomycetes.

Sex may be taken as equivalent to karyogamy, the formation of a hybrid zygote from the fusion of two intact "gamete" nuclei. Peripheral to this essential, sexual processes are subject to a variety of classifications based on discordant criteria, giving such distinctions as: isogamy versus heterogamy; monoecy versus dioecy; prezygotic versus postzygotic meiosis; syngamy via conjugation versus copulation versus persistent dikaryophase; germinal versus somatic reduction; meiotic versus mitotic crossing over; autogamy versus exogamy; and many others. The initial act of karyogamy (hybridity) must also be distinguished from chromosome segregation or elimination and from crossing over within chromosome pairs as aspects of sexual recombination cycles.

Among bacteria, the genetic analysis of sex has been carried furthest with *Escherichia coli,* some 5% of the strains tested so far being fertile (Lederberg and Tatum, '53). In addition, similar methods have been applied by various authors to support their tentative claims of sexual recombination in *Achromobacter fischeri* (McElroy and Friedman, '51), *Serratia marcescens* (Belser and Bunting, '54), and *Bacillus megatherium* (Delamater, '53), but not in *Proteus* L forms (Hutchinson and Medill, '54), *Azotobacter agile* (Ziebur and Eisenstark, '51), *Pseudomonas fluorescens* (Lederberg, unpublished), or *Salmonella* (Lederberg, '47b; Zinder and Lederberg, '52). Experiments with *Streptomyces griseus* have been indecisive owing to confusion from heterokaryotic interactions (Lederberg, unpublished), but a tentative suggestion from morphological studies (Klieneberger-Nobel, '47) on the sexual origin of the entire aerial mycelium has no genetic support. Other claims of bacterial sexuality based on suggestive photographs deserve closer genetic attention than has so far been recorded.

### *Genetic transduction*

Although the preceding mechanisms are important in relating the fundamental genetic structure of microbes to higher forms, their exposition does not give much leverage on the mechanism of crossing over, which is the fulcrum of this symposium. However, recombination in a number of bacterial species has been found to occur by genetic transduction, a new mechanism which differs from sex by the fragmentary nature of the unit of exchange. That is, transduction is defined as the transmission of a (nuclear) genetic fragment from a donor cell (which in every case so far is destroyed in the process) to a recipient cell which remains intact.

Like sex, transduction may be classified by several criteria. Rudimentary knowledge already distinguishes at least two categories, depending on the agency of transfer: deoxyribonucleic acid (DNA) (pneumococcus, Avery *et al.,* '44; *Hemophilus influenzae,* Alexander and Leidy, '51) or carriage by a virus particle *(Salmonella,* Zinder and Lederberg, '52; *E. coli,* Morse, '54). Other categories might depend on the frequency of transmittal, the specific characters that may be or have been transmitted, the persistence of the intermediate heterogenic state, the complexity of the fragments, or the bacterial species. There is little or no indication of transduction in higher forms, but too few experiments have been reported (see Marshak and Walker, '45; Mazia, '49; Klein, '52) to be conclusive in the face of the obvious technical obstacles. Possible further examples of transduction that have not been so fully analyzed are reviewed elsewhere (Lederberg, '48, '49a; Austrian, '52).

Probably inadvertently, a previous discussion in this symposium may have intimated that transduction in the pneumococcus (type transformation) became relevant to recombination only after two or more markers were explicitly followed, and their reassortment noted. But Griffith's experiment ('28) already posed a serious genetic question: How does the unit recombine with the whole? During the following two decades,

the chemical analysis of pneumococcus transformation took precedence over genetic consideration. Some tentative suggestions were adopted that failed to encourage multiple-marker experiments (or were based on their absence) and were therefore barren — "directed mutation" (Dobzhansky, '41) or infection by a presumably cytoplasmic "virus" (Lederberg, '49a, among others). The concept of transduction to explain the pneumococcus transformation and succeeding examples was, however, well stated by Muller ('47): "still viable bacterial chromosomes, or parts of chromosomes . . . might . . . have penetrated the capsuleless bacteria and in part at least have taken root there, perhaps after having undergone a kind of crossing-over with the chromosomes of the host." Most of the genetic analysis that succeeded this prescription has been based on this point of view, and its success has amply justified the concept.

In the pneumococcus, transduction is mediated by raw DNA, extracted from bacteria that are fragmented with bile salts, and refined by the chemist (McCarty, '46; Austrian, '52). Much insight into the chemical constitution of the genetic reagent has been achieved, and a plausible case has been presented for the sufficiency of deoxyribonucleate alone (as it has for the genetic content of phage). For so crucial a question, however, the standards of proof should be more than ordinarily rigorous (Hershey, '53), and some obstinate doubts on the possible accessory role of protein components will be dissolved only when the non-DNA residues of the preparations are shown to be stoichiometrically disqualified. This standard is admittedly as high as or higher than any in biochemistry but is commensurate with the stature of the conclusions. We have to keep in mind the difficulties in the physicochemical characterization of linear polyelectrolytes which vitiate such criteria as electrophoretic or sedimentational homogeneity, as well as estimates of particle size.

In *Salmonella*, on the other hand, the genetic fragment is embedded in a phage particle, from which it has not been extricated in active form, possibly only because we have not

learned to imitate the faculty of penetration by the virus into the new host cell. The fragment is, at any rate, inaccessible to deoxyribonuclease or any other informative reagent, but it would be plausible to equate the genetically identified fragment in *Salmonella* phage with the DNA preparations from the pneumococcus. Although we are thus hindered in chemical studies of *Salmonella* transduction, we can console ourselves with the possibility of some understanding of virus biology, and with the merely technical advantages of *Salmonella* for genetic research.

Details of the relation of the genetic fragment with the maturing phage particle were discussed by Zinder earlier in this symposium. To review very briefly, transducing activity has been detected in lysates of *Salmonella* species roughly in proportion to the number of phage particles (Lederberg *et al.*, '51; Zinder, '53; Zinder and Lederberg, '52). Some means of selective isolation is always needed to detect the altered cells, for any given trait is transduced by about one per million phage particles, and the number of phages that can be effectively adsorbed by a single bacterium is limited. The competence, i.e., the range of traits that can be transduced by the various particles in a given phage preparation, is rigidly determined by the genotype of the host cells. Every character that has been tested is subject to transduction, with only second-order differences of efficiency as described by Zinder. These characters include nutrient requirements, sugar fermentations and inhibition, antibiotic resistance, motility, and flagellar antigens. The active material in the lysates is identified with phage (as carrier) not only by surface resemblances in numerical proportionality to plaque count, size (gradocol filtration; sedimentation), tolerance to heat and disinfectants, adsorption on various bacterial serotypes, and neutralization with antiphage serum, but also in the correlation of transduction with virus infection and lysogeny at low ratios of phage: bacterium. This shows best that the same skins enclose phage and fragment. These may be differentiated, however, by the use of ultra-

violet light (the fragment showing a much smaller cross section than the infectivity) or by the use of bacterial hosts to which the phage is not adapted and in which it fails to proliferate. Finally, it should be noted that transduction is mediated in much the same fashion by "temperate" phage grown in the lytic cycle, "temperate" phage obtained by ultraviolet induction of lysogenic bacteria, and "lytic" phage mutants (necessarily grown in the lytic cycle and applied to lysogenic, immune recipients).

### *Lysogenic conversion*

A lysogenic bacterium has been understood to be distinctive in its hereditary makeup since the early investigations of Burnet ('34) and others, but the preconception of most geneticists (including Lederberg, '49a) doubtless favored a cytoplasmic localization of the latent, symbiotic virus. The sexual system of *E. coli* K-12 permitted the first explicit investigation of the genetics of lysogenicity (Lederberg, '51; Lederberg and Lederberg, '53; Appleyard, '53; Wollman, '53) which showed that this trait, far from depending on exceptional cytoplasmic factors, rested on the same basis as the other mutually linked genetic determinants of the bacterium. The penetration of the "temperate" phage lambda into a sensitive host bacterium is thus followed either by bacteriolytic multiplication of the phage, or by the incorporation of the genetic material of the phage into the bacterial chromosome at a specific locus, *Lp*, closely linked to *Gal* (galactose fermentation). This virogenetic locus is reproduced *pari passu* with the remainder of the bacterial genotype. In some of the lysogenic descendants, it may again become autonomous, to reinitiate the bacteriolytic cycle and the release of infective virus. It is not yet clear whether the virogenetic segment simply adheres to the homologous locus or actively replaces it. Some analogies with transduction involving the same phage suggest that both occur in sequence, which may also explain Appleyard's "double lysogenics" ('53).

In either event, should lysogeny be characterized as a species of genetic recombination? To traduce Hershey ('53), the bacteriophage particle can be considered as a miniature, somewhat simplified bacterium with an outer membrane or skin and an internal nucleus (DNA or vegetative phage). When a host bacterium is attacked, the skin and tail of the phage are left behind and the nucleus penetrates (as in the fertilization of egg by sperm) to initiate the developmental cycle of infection or lysogeny which will ultimately result in the reappearance of infective (complete or mature) phage particles. Lysogeny consists of a strikingly intimate union of the phage nucleus with the bacterial genotype. We have the arbitrary choice of defining the lysogenic bacterium (in common with other symbiotic complexes; J. Lederberg, '52) as the association of two organisms, or as a novel recombination having a good deal in common with fertilization or transduction. The recombination frequently results in alterations of bacterial behavior having to do with host-virus interrelations (Luria, '53; Lwoff, '53; Boyd, '54). But it may also result in more insistent changes of bacterial qualities that would not at first sight have been related to a virus: toxin formation in the diphtheria bacillus (Groman, '55), colonial morphology in *B. megatherium* (den Dooren de Jong, '31; Ionesco, '53), and somatic antigen in group E *Salmonella* (Iseki and Sakai, '53; Uetake *et al.*, '55). In this respect, these lysogenic conversions resemble the transduction cited, but the alterations here are inseparable from lysogenicity, i.e., the genetic quality is specifically associated with the phage nucleus, not a desultory companion.

This concept of lysogenicity implies that the incorporated phage nucleus now functions as a segment of a bacterial chromosome (Lwoff, '53). The conversions might even be represented as atavistic remnants of the bacterial functions of such segments before their differentiation. Indeed, the phylogeny of any virus cannot be safely argued, since primary vestige cannot be distinguished from secondary adaptation of the parasite. It therefore cannot be said whether

the virus has evolved from the bacterial segment become suddenly autonomous, or whether the incorporation of the segment is the extreme of parasitic specialization. In fact, we should not be too complacent that the latent virus is always embodied in the bacterial chromosome lest differences be overlooked in other systems that might lead to a broader perspective.

## TRANSDUCTION AND RECOMBINATION

Genetic transduction may be divided into its initial and terminal phases, the fragmentation of the genetic material, the introduction of fragments into a new cell and their incorporation in the genotype. As far as present information is concerned, the fragmentation is essentially random, although Zinder's data show differences (possibly topographic) of timing in the assumption of different fragments into mature phage. Unfortunately, since nothing is known of the localization of latent virus in *Salmonella,* random assumption cannot be related to what might be an equally indeterminate intrabacterial site of virus fixation or growth. Linked transductions (Ephrussi-Taylor, '51; Hotchkiss and Marmur, '54; Leidy *et al.,* '53; Stocker *et al.,* '53) show that the fragments are not "single genes," but it cannot be said from these experiments whether "crossing over" of linked factors results from initial fragmentation or a later differential implantation (or both). In the DNA-mediated transductions, we can ask whether more gentle preparative methods might preserve otherwise broken associations, but information on possible limitations on the size of effective particles is lacking. But at least in a qualitative way, we can readily visualize how chromosomes can be fragmented without destroying the vitality of the parts: this is a familiar intracellular experience in radiogenetic work. We can also speculate how (or accept the fact that) such fragments are introduced into a new bacterium. But how shall we understand incorporation? A view once stated (Lederberg, '49a) that "from purely mechanical considerations it would seem most likely that the

transforming agents are incorporated into a cytoplasmic system like that of *'kappa* . . . a parallelism with induced lysogenicity'' was based on the incorrect premise that ''the more credible reports uniformly picture the *acquisition* of a genetic function'' and the lack of data on factors other than the capsular polysaccharide. Of course transduction may involve any element of the entire genotype and entail the replacement of the homolog, not merely an addition of a genetic factor. This is especially well shown in the substitutions of alternative (multiple) alleles for the flagellar antigens in *Salmonella* (Lederberg and Edwards, '53) which have been carried back and forth repeatedly. After transduction, the allele that had been replaced could not be detected by either phenotypic or genotypic (transductive) analysis. There is, therefore, a problem of integration, not just addition. It is difficult to see how a cytoplasmic system, that is, a genotype as dis-integrated in the living cell as it is in a DNA preparation or a phage lysate, could meet the demands of genetic stability, and even more so to envisage mutual replacement on this scheme.

Fortunately, a new transduction system involving *E. coli* K-12 and lambda (Morse, '54), furnishes some tangible facts to bolster these a priori doubts. This system differs from *Salmonella* insofar as the only genetic factors so far found to be capable of transduction are a cluster of closely linked loci ($Gal_1$, $Gal_2$, etc.) concerned with galactose fermentation. This cluster is also linked with *Lp,* the locus of fixation of lambda in the lysogenic bacterium. The second important difference is the persistence of the heterogenic state; that is, the transductions lead to clones that are apparently ''heterozygous'' for the *Gal* factors involved. The heterogenic bacteria later segregate to give either of the two parental forms (with respect to the *Gal* factors) or, more rarely, crossovers. In this species, therefore, introduction and incorporation are separated in time, and can be more readily analyzed. A given

heterogenic clone permits crossing over between the introduced fragment and the intact genome, with different results in different cells. Sexual recombination analysis, especially the segregation behavior of diploid hybrids, assures that the *Gal* and *Lp* loci are normally integrated (Lederberg and Lederberg, '53) into the linkage system. This provides a partial answer to the previous question on the timing of crossing over in transduction of linked factors, but the incorporation or crossing over of fragments that Muller had visualized ('47) must still be detailed.

Here we face the dilemma of this symposium: Shall we adhere to a mechanical breakage picture, with its obvious difficulties in the postulation of precise double breaks, with the even greater improbability of double crossing over? Or shall we dispose of familiar difficulties by an appeal to the unknown, by postulates of the mechanism of genetic replication? As long as facts and fancies are clearly separated, some speculations may be in order.

Following Belling's lead ('33), we may be strongly tempted by one or another copying-choice principle in connecting crossing over with chromosome reproduction rather than chromosome breakage, as illustrated in figure 1, which begins with the fragment introduced into a new cell. We should not balk then at postulating its synapsis with the homologous element: it is inconceivable that replacement could occur without specific pairing of some sort. The next steps are more obscure, but the end result is an effective double crossover between the fragment and the intact chromosome. Sequence A shows two pairs of breaks, on the mechanical theory. Sequence B shows Belling's theory, with a choice of interconnections after reproduction of the elements; sequence C is very similar, with a choice in the models for reproduction of the new chromonema.

The copying-choice models (B and C) may also be applicable to other enigmatic examples of frequent double exchange within limited regions, such as the fourth chromosome in

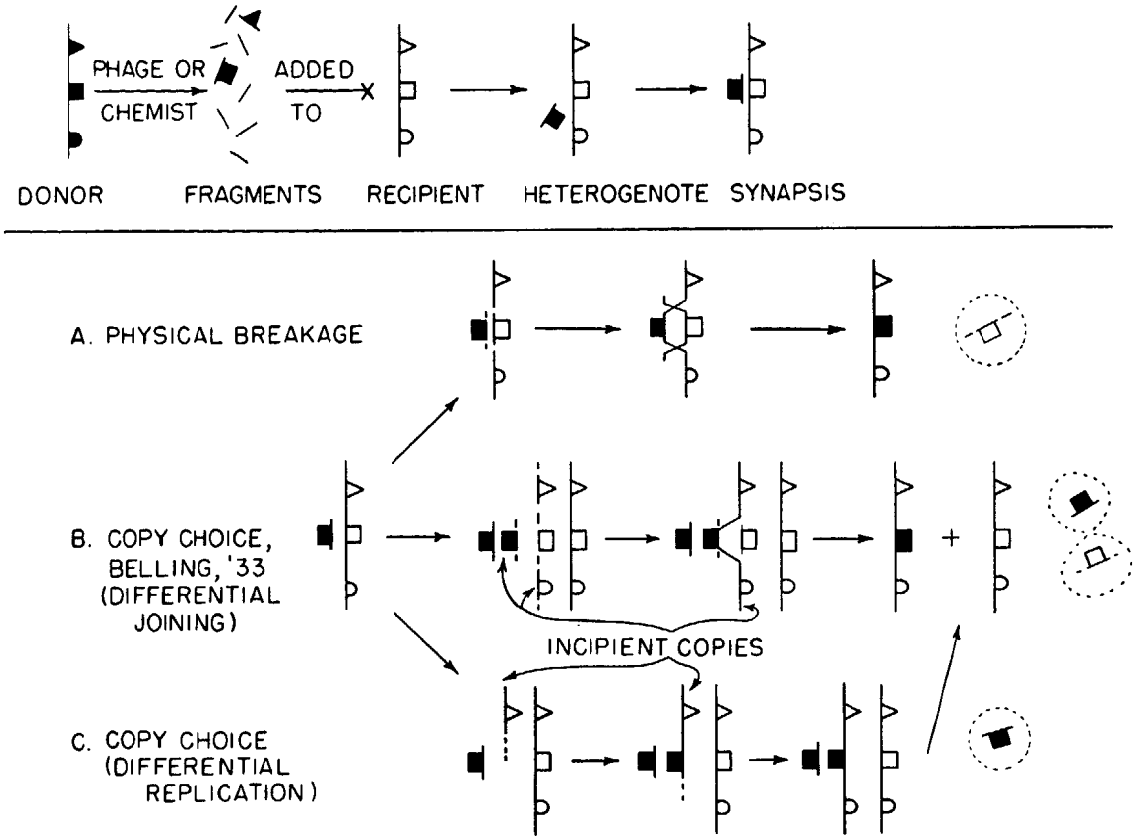


Fig. 1 Hypothetical schemes for the incorporation of transduced fragments. The top line shows the preliminary stages of fragmentation and reintroduction of fragments into recipient cells, common to all three proposals. The fate of the residual fragments, enclosed in dotted circles, is unspecified.

*Drosophila* (Sturtevant, '51), pseudoalleles [2] in *Neurospora* (Giles, '51), maize (Laughnan, '52), and *E. coli* (E. Lederberg, '52), and to the "conversions" in yeast heterozygotes (Lindegren, '53). So far as I know, there are no experimental data on the possible incorporation of small acentric fragments artificially produced at the appropriate stage of meiosis in higher forms, and I would leave to Novitski and McClintock the problem of engineering and interpreting such an experiment. Nor, so far as I know, have intercellular transductions been explicitly attempted with organisms that would be amenable to detailed cytogenetic analysis (some early trials with *Neurospora* auxotrophs were negative or confounded by spontaneous reversion, Ryan and Lederberg, '46).

It is not immediately apparent how these hypotheses can be tested experimentally. Some information might be had from closer study of the immediate progeny of transformed cells, but there are many technical difficulties. To Hotchkiss' account in this symposium one might add that his organism is a *Diplococcus,* and that the units of plating experiments are typically pairs of cells, at least. But this difficulty is not unusual, only more obvious in his organism. Most bacteria have several nuclei within each cell, with the same effect. For further cell lineage studies on genetic replication, mutation, and transduction, it would be indispensable to have a

[2] Alternatively, one can envisage *Ds*-like transpositions from one chromosome to the other along the lines of McClintock's observations ('51). The bearing of *Ds* and other position effects on pseudoallelism (Laughnan, '52) deserves reemphasis, especially where the *cis* and *trans* heterozygotes differ. In maize, the insertion of *Ds* simulates mutation at nearby loci; presumably such insertions need not be all precisely isblocal, so that either crossing over, or other means of loss of *Ds* might restore the normal condition in crosses of recurrent mutants. Since gene localization can be studied only with mutations, our concept of a locus as a site of primary genetic function cannot be independently validated, and it may be meaningless to distinguish between a gene and the loci of nearby modifiers. This picture offers no support for the insistence on the origin of pseudoalleles by duplication, or for the notion of structural complexity, separable by crossing over, within genes unless a locus is redefined as a region within which characteristic end effects are generated. Recent reports suggest, moreover, that we shall have to be reconciled to "pseudoalleles" as a feature of any locus that is studied with sufficient diligence.

uninucleate organism whose cytogenetic status could be confirmed by both cytological and genetic means. This would still leave the possibility of confusion from polyteny, a consideration that also arises from other sources described in a later section.

## SOME RECENT FINDINGS ON SEXUALITY IN *E. COLI*

This subject was comprehensively reviewed three years ago (Lederberg *et al.*, '51). At that time, "*E. coli* K-12 is recorded as a homothallic system, for no preferential compatibilities have been found in recombination experiments involving a wide range of mutants derived from K-12. In particular, no segregation of oppositional compatibility factors could be detected from persistent diploids, in contrast to the . . . mating type mutations in *Schizosaccharomyces pombe*. Preferential compatibility would be very useful for further analysis, and is carefully looked for especially in crosses involving new strains."

Had we waited, it was to have been found among newly isolated fertile strains, but not long after this quotation was recorded, a compatibility system was discovered within the K-12 strain also (Lederberg *et al.*, '52; Cavalli *et al.*, '53). We learned that some sublines of K-12 were compatibility mutants, symbolized $F-$. Crosses of $F- \times F-$ are completely sterile. $F+$ (the wild-type state) $\times F+$ is fertile, and $F+ \times F-$ even more so. Most of the crosses of previous experiments were $F+ \times F-$: why was the compatibility system not discovered earlier? It turned out that the progeny of these crosses did not segregate, but were uniformly $F+$, and that mere contact of $F+$ with $F-$ cells in mixed culture was sufficient to convert the latter to the genetically stable $F+$ state. Therefore not until two distant $F-$ "mutant" clones had been tried against each other, or an $F-$ subline tested for self-compatibility, could the system be detected.

Meanwhile, Hayes ('52) was studying the effect of streptomycin on fertility, and by good fortune, worked with a pair

of stocks that were identifiable as $F+$ and $F-$. His discovery of a residual sexual fertility in one of these stocks ($F+$) after treatments that left a negligible number of viable (colony-forming) cells was therefore promptly related to the compatibility system, and has helped to illuminate it. But I am unable to concur that this experiment speaks for the participation in the sexual process either of "genetic elements extruded by the viable cell which adhere to the cell wall" or of the virus (lambda) inherent in lysogenic strains of K-12. The latter had already been ruled out by the full fertility of nonlysogenic parents (Lederberg, '51), and the former finds no support in the abject failure of the most assiduous efforts to separate subcellular agents that would function in "sexual" recombination (Lederberg, '47a; Atchley, '51; Davis, '50). The streptomycin effect does show at least a physiological distinction between the two parents, and would have an easy explanation if the zygote received most of its cytoplasm from the $F-$ parent, and were fertilized without gross contamination by the streptomycin-inhibited substance of the $F+$ cell, that is, if mating involved conjugation rather than copulation.

[*Note added June 1954:* Direct support for this picture has recently been obtained by microscopic experiments with very actively mating (*Hfr* and $F-$) cultures, in which one parent is from a motile strain, the other nonmotile. Within an hour of mixing, I find pairs consisting of one cell of each parental line. The pairs are joined laterally and are readily discerned owing to the disparity in motility. After another hour or so, they disjoin. With the micromanipulator, exconjugants have been isolated and permitted to form clones. Usually, both remain viable, and recombinants are found with very high frequency among the progeny of the $F-$ cell. It is therefore concluded that the conjugation permits the transfer of a gamete nucleus from the *Hfr* to the $F-$ cell, followed by karyogamy and meiosis.]

Perhaps the most obscure feature of the K-12 system has been the aberration from mendelian segregation of unselected markers. In the earliest experiments, this was partly ob-

scured by the necessity of selecting certain combinations of markers, usually auxotrophic, in order to detect rare recombinants, but it is equally a feature of crosses where selection can be relaxed owing to the high frequency of recombination. The aberration consists of a relative bias in favor of markers from the $F-$ parent. This has led Watson and Hayes ('53) to suggest that the $F+$ gamete, which is, according to their version, morphologically subcellular, is also defective with regard to one or more chromosomes. This hypothesis of gametic or prezygotic exclusion is not readily distinguished from the alternative, of postzygotic elimination, by consideration of the haploid recombinants only. The biases would clearly be similar whether the genetic contributions from the $F+$ parent were lost before or after the zygote was initiated.

The aberration is seen in the most clear-cut qualitative fashion, however, in the behavior of nondisjunctional diploids (Lederberg, '49b) which occur with highest frequency among the progeny of so-called *Het* mutant stocks. These diploids also show strongly aberrant segregation ratios for markers which are heterozygous, so that this cannot be attributed to prezygotic exclusion. Moreover, they are regularly hemizygous for a pair of linked factors, *Mal* (maltose fermentation) and *S* (streptomycin resistance) though diploid for some fifteen or twenty others. The deficiency for this segment would be sufficient to explain the aberrant segregation, since it should act as a haplolethal and prevent the recovery of any allele linked to it except as coupling is broken by crossing over (Lederberg, '49b; Lederberg *et al.*, '51). Does the deficiency arise by a gametic or a postzygotic process? Closer consideration of the diploid types supports the latter.

When the diploids were first isolated, the hemizygosity was quite perplexing but even more so was the bias with regard to its polarity. In any given cross, most of the diploids were hemizygous for the *Mal* or *S* marker(s) of one parent, but some carried the other. Among the diploids, such a bias could no longer be attributed to linkage to nutritional factors, and no other basic distinction between the parents had been recog-

nized that could account for nonrandom loss of the alternative segments. Nevertheless, since some diploids retained the full set of markers from one parent, and others from the other, the lack of any complete diploids (i.e., heterozygous for *Mal, S*) suggested that the elimination occurred regularly after the zygote had formed. Otherwise, one would expect the union of complete gametes to result occasionally in a complete diploid. A few examples of amphitypic diploids, carrying, e.g., the *Mal* allele from one parent, *S* from the other (Lederberg *et al.*, '51, table 6) also suggested that crossing over preceded the elimination.

A more thorough reinvestigation (Nelson and Lederberg, '54) has confirmed this inference. Persistent diploids were isolated from $F+ \times F-$ crosses differing only in their *F* polarity, and tabulated in regard to *Mal* and *S*. Each of the 635 diploids tested was hemizygous for *Mal* and *S*, but regardless of the parental polarity, about four-fifths carried the alleles from the $F-$ parent, about 15% from the $F+$ parent, and the remainder were amphitypic. Thus the elimination must be postzygotic, but must preferentially involve the segment that had been introduced from the $F+$ parent (in the light of later experiments, that is, the migratory nucleus). To account for the incomplete determination, it may be speculated that at meiosis a single locus always breaks on the $F+$ chromosome, but that prior crossing over occasionally saves one of or both the $F+$ markers with a corresponding loss of their opposite numbers from the $F-$ parent.

None of this sounds as if it could be fundamental to a sexual cycle, and if so it might be better to search for more straightforward patterns in other strains of *E. coli*. Some strains at least appear to function independently of the $F+$ agent, though they can be "infected" with it. So far, with these strains, such "infection" can be detected only by carrying the agent back into a K-12 line tester stock. Without the good luck of diploid analysis, however, it requires the most tedious development of stocks and study of crosses to study

the basic segregation patterns of new strains, so this is neither the first nor the last time this hope will have been voiced.

Almost no progress has been made on the nature or transmission of the *F* agent. The rapid contagion in mixtures of *F* + and *F* — cells has been mentioned, but this is not paralleled by successful "infection" with cell-free preparations. For example (T. C. Nelson, unpublished), converting mixtures of *F* + and *F* — cells have been poured within a few seconds through membrane filters directly into susceptible *F* — cultures, without the least alteration of the latter. Perhaps, the transmission of the clonally stable *F* + state requires the direct superficial contact of two cells; at any rate, if there is a virological problem at all it may be analogous to the plant viruses which have so far defied artificial transmission.

## SINGLE CELL PEDIGREES AND TRANSDUCTION IN *SALMONELLA*

For pedigree analysis it is especially rewarding to follow traits that can be determined in single cells. The morphological differentiation observable in living bacteria is so limited that the character of motility stands almost alone for this purpose, but has proved to be most useful. When a nonmotile mutant of *Salmonella* is exposed to appropriate phage lysates, 1 to 10 per million cells can be provoked to give motile clones. Macroscopically, these are readily selected by platings on a soft gelatin-agar (Hiss, 1897; Colquhoun and Kirkpatrick, '32), on which the nonmotile culture is restricted to the site of inoculation, but through which motile bacteria readily swim as they proliferate, to form progressive cloudy swarms. In addition to the conspicuous swarms, however, Stocker *et al.* ('53) also described trails or chains of small colonies that might extend 10 to 20 mm into the agar. We concluded that the trails represented an abortive transduction, whereby a genetic factor was transduced to a nonmotile cell in a form capable of restoring motility to the recipient, but incapable of reproducing with it. The transformed cell would therefore divide to give one motile and one nonmotile daughter. The

former would continue to move, the latter would (rather promptly) stop and, by forming a colony in situ betray the trail of the cell. Since the trails were definitely unbranched, at least in the terminal portions that could be carefully examined, we concluded that the metapoietic particle did not reproduce at all during a hundred or more bacterial generations. Sooner or later, the trails terminated, presumably from some accident; there was no indication of a swarm issuing from a trail. Subsequent micromanipulation experiments both here and in Stocker's laboratory at London have provided a new approach to the problem.

If mixtures of nonmotile bacteria and competent phage are planted in an oil chamber, and examined with the microscope, motile cells begin to appear after about 2 hours' incubation. Unfortunately, as many as two or three divisions may take place during this interval, which truncates and complicates our genealogies. The motile bacteria can, however, be readily trapped when they are permitted to swim into adjoining empty droplets, and thus can be isolated one by one. In the system I have worked on (TM2 — × SW-666), the incidence of motile bacteria is rather low, but their viability fairly good. About 10–20% of the isolated bacteria die before engendering sizeable clones. About 5–10% give rise to clones containing anywhere from 25 to 100% of motile cells. The fraction of segregating clones would presumably be higher were it not for the initial bacterial divisions. These motile cells are evidently stable transductions: they engender only motile progeny, and are thus equivalent to swarms. The nonmotile sibs have so far all been parental, none complementary crossovers (with regard to the antigenic factors linked to motility in this transduction), nor has more than one antigenic type been found in a given motile clone.

The remainder of the motile cells are trail equivalents, that is, they give progeny whose motility follows the law of primogeniture, as had been hypothesized from the appearance of the trails in agar. To simplify the following discussion, let us call a cell (or cell lineage) a semiclone if it persistently

transmits motility to just one descendant through several fissions.

The outstanding discrepancy between the microscopic results and inferences from the trails is that a single isolated motile cell may engender during the first five to ten fissions not just one, but up to about 100 semiclones. The discrepancy probably arises from delay in the penetration of motile bacteria into the agar, and their orientation away from the inoculum, until after these early divisions. But after this early interval of apparent "replication" of the motility factor, strict semiclonal behavior is followed until, for reasons unknown, motility is terminated. So far, semiclones have been followed up to 59 fissions, but are usually seen to terminate earlier, often by 20 or 30. The length of time, and the number of progeny involved, have obviously made it impossible to follow any single clone in its entirety ($2^{59}$ bacteria would weigh 50 tons!) and this picture has been reconstructed from observations on many motile individuals repeatedly reisolated from different clones of different sizes and at various times. Separations of early fissions show, however, that a cell may divide to give one cell a swarm equivalent; its sib the parent of several semiclones. Also, in clones containing large numbers of semiclones, the split during early divisions is grossly unequal: at the 4- or 8-cell stage, one may give 100 semiclones, another less than 10, another none (detected). This rules out any random partition of elements.

How can all this be interpreted? Three hypotheses, which may each have numerous modifications, have been suggested:

(1) The semiclones represent, as originally postulated, the transduction of aborted genes with a limited capacity for irregular replication. This not only fails to account for the sharp transition between the early and later behavior but the *ad hoc* resort to "irregular replication" discourages further study.

(2) The semiclones represent "genes" that are now totally incapable of reproduction owing either to their position or prior accident but still capable of functioning. The multi-

plicity of semiclones represents a degree of polyteny in the bacterial chromosome, and the early divisions serve to distribute the units to the progeny. A cell with but one unit is a semiclone. But what an extraordinary degree of polyteny! And one is surprised that the unit is never incorporated to re-form a motile clone.

(3) The reproductive incompetence of the units is not accidental, but characterized them in the intact donor cell as well. That is, the units are not genes in fact, but the primary products of genes organized in complex bundles. The bundles would perhaps be closely associated with their genic source, but are separated in transduction.

None of the indicated objections to any of these hypotheses is fatal, and we have no certain means of choosing which, if any, is correct. For example (as suggested by Sonneborn), a sterile genetic fragment might be transduced that was still capable of producing the primary products. These units would then initiate the semiclones. Still other hypotheses are imaginable. For the moment, number (3) seems the most fruitful in suggesting further experiments; for example, it attempts to correlate functional status of donor cells with yields of semiclones.

These remarks are presented for two reasons — to elicit further constructive suggestions on interpretation, and to emphasize the value of going back again now from the statistical, populational methodology in microbial genetics to a respect for the individual cell.

## DISCUSSION

*Chairman* HERSHEY: I should like to bring up again the question that Dr. Lindegren raised this morning; namely, how do you distinguish between linked recombination and transduction in a cross using lysogenicity for one marker and a transducible character for the second? As I understand it, this confusion might appear in the historically important case of $Gal_4$ and the carrier state for lambda in K-12. I think it might be useful if Dr. Lederberg would clear this up.

LEDERBERG: To answer Dr. Lindegren's question first, we would certainly have been confused if transduction had occurred together with sexual recombination. However, the transduction in K-12 involves only a single group of markers concerned with galactose fermentation, and could be neither discovered nor confusing until these were studied. Transduction and sex can be isolated from each other by the proper choice of stocks and conditions. Transduction is mediated by a phage which is readily filterable; the filtrates contain nothing that will function in place of the intact cells in sexual recombination. Also, $F- \times F-$ crosses are sexually completely sterile; nevertheless, Mrs. Lederberg has shown that this incompatibility does not hinder transduction by phage, but again, this is limited to *Gal* factors. On the other hand, sexual interaction takes place unhindered though both parents may be nonlysogenic, or if both carry the $Lp_2^r$ mutation that prevents the adsorption of lambda, although either condition naturally prevents transduction from being effected.

I may add that we have not found deviations from qualitative regularity in segregations from diploids (heterozygous for $Lp^+Gal^+/Lp^sGal^-$ as well as a host of other markers) that would be called conversion of one chromosome by another. But we lack Dr. Lindegren's advantage of tetrad analysis. It is obvious that incorporation by copying choice could be modified to fit the conversions that he has described, and which deserve the most careful attention.

Dr. Hershey asked about the bearing of transduction on the genetics of lysogenicity. Something was said about this in my talk, but we do not have all the answers yet. However, we still find the clear-cut linkage of *Lp* to *Gal* in crosses where transduction is ruled out as indicated before. In any event, transduction occurs with an efficiency of about one per million phages, which is incomparably lower than the segregation ratios of *Lp* and *Gal,* and would not account for the incidence of both parental couplings in crosses. Since there seems to have been some misconstruction in recent reviews, I want to emphasize that these are quite distinct, though closely linked,

loci and that, while the numerical segregation ratios are strongly biased in *Hfr* × *F* — or *F* + × *F* — crosses, both parental and recombinant classes are found among the progeny. Another useful criterion is the initial heterogenic instability of the transductions; this has never been seen among recombinants. (We are now setting up crosses with the heterogenotes to look for some evidence on the association of the transduced fragment with the homologous segment of the intact genome.)

For a time there was some question about the interpretation of the *Lp-Gal* linkage in the light of the segregational aberrations of *F* + × *F* — crosses. I believe this doubt is no longer current; at any rate, there has been no suggestion as to why *Lp* should be "pseudolinked" to *Gal* any more than to any other marker, e.g., *Mal*. But one could even pass over questions on the details of zygote formation and examine diploids heterozygous for these and other markers. The concordant, linked segregation of *Lp, Gal,* and all other markers from these diploids is the most compelling evidence of the chromosomal basis of lysogenicity.

ATWOOD: If your semiclones are caused by a nonreplicating product that would stick . . . ?

LEDERBERG: I do not know where they stick. All I know is that they are in the cell. There is no indication as to the localization of the particles that I am talking about here in the bacteria that have been transformed.

ATWOOD: In any case, if they are the result of a nonreplicating product that can function independently of the gene which produces it, then you ought to get semiclones not only following transduction, but also whenever there is a mutation to nonmotility.

LEDERBERG: That is a point I should have made. One should look, in all experiments of this kind, not only for permanent genetic alterations but also phenotypic modifications. That would apply particularly to the pneumococcal and other case provided you had markers where that sort of thing could

be detected. So far, motility is almost the only one that will work, where the phenotype of an individual cell can be diagnosed; and when there is little enough secondary phenotypic delay, as appears to be the case here, not to obscure the results on that basis.

As to whether one should always get this phenomenon, that depends on the hypothetical relation of the products to the gene. It is possible that they are regularly bound to the gene — at least the larger number of them. Then you would get semiclones only under circumstances which would disrupt that relation, namely, in transduction.

However, one or two nonmotile stocks occasionally do give a trail as well as swarms by spontaneous reversion.

But of course even the spontaneous trails could just as well be explained by the other two hypotheses since they could represent cases where the genetic material has been damaged to such an extent that it cannot reproduce.

STENT: It seems to me that if you admit cytoplasmic fusion in the case of recombination, then the role of virus as the possible agent is not entirely excluded.

LEDERBERG: I did not say it was.

STENT: You seemed to think that the *F* agent could not be a virus.

LEDERBERG: I think any group of medical bacteriologists would have slaughtered me if I had tried to give this kind of evidence for the existence of a virus. We should be as careful in defining a virus in this area as in others. We have a contagion phenomenon and would like to find a virus to explain it, since we have no other way to do it, but it has not been found.

STENT: Are no lysogenic viruses known that could identify the virus as acting like the *F* agent? Since transduction is known to be a phenomenon that can occur with virus, then indeed under the concession that the *F* agent is a virus, an understanding of the phenomenon would be advanced. At least it would be unified.

LEDERBERG: Our understanding of the phenomenon will be most advanced when the *F* agent is isolated from the cells so that its genetic properties can be described.

STENT: But it cannot be isolated because, if the *F* agent is defective prophage that never achieves maturity, i.e., exists only in the cells in the vegetative form, then any attempt to break up the cell would immediately destroy it.

LEDERBERG: It does get over from one cell to another.

STENT: Yes, through cytoplasmic fusion.

LEDERBERG: I think we are now talking about words. I would describe such an element as the gamete of the bacteria, and would then proceed to do experiments to determine the genetic content of that gamete; those that have been done indicate that the zygote that is formed is complete. If you want to attribute virus-like properties to these agents, too, then you must think of experiments to settle the problem. But I think that, until it is isolated, we had better be careful about assuming it is a virus, because the impetus for trying to isolate it might be lost. I consider that to be the most important question in that particular area. The results have been so uniformly negative that we might well be suspicious of that negativity and begin trying to think of things other than viruses to explain it.

It is quite conceivable that in a situation where a virus-like agent — or call it an *F* + agent — is necessary for the effective contact between, say an *F* + and an *F* — cell, that agent may be what bores the hole in the *F* — cell. There are any number of possibilities. But in this part of the story where there are some facts, I preferred to stick to them.

In spite of the temptation to speculate, the facts are that, in order to get a mating, one of the parents must be *F* +, and presumably a surface property of the bacterium is altered. However, the *F* + agent, this thing that is capable of converting, is not by itself a sufficient condition for the *F* + property of compatibility. It is not even a necessary condition since there are compatible cultures *(Hfr)* that cannot convert. Aeration of an *F* + culture produces a population of cells,

every one of which has that agent, because they can give rise to clones which are capable of converting, but none of which have the property of genetic recombination.

This is why I have not wanted to be too specific too soon about the possible effect of the $F+$ agent, and prefer to say that it has an effect on the surface of the cell rather than to say that it itself is the character. We are really back to the old story of the relation of gene and character. Here is at least one criterion by which they can be separated; namely, this aeration where there is still the heredity of $F+$, but not its action, in those cells.

STENT: I think the distinction perhaps is more than words because it would explain the streptomycin effect, which I understand is unexplained under your conception.

LEDERBERG: You probably misunderstood my discussion on that point. The streptomycin effect, as we now understand it, does strongly suggest that the cytoplasm of the zygote does not receive a very large contribution from the cytoplasm of the cell that has been steeped in streptomycin, but that is all that one needs to postulate.

On the notion of a conjugational type of exchange of nuclei and a limited amount of cytoplasm, too, if you like — we have no criterion for it — that problem is completely solved.

STENT: Why does treatment of the $F-$ cell with streptomycin cause infertility?

LEDERBERG: For the reason that I have just indicated, that on this notion the larger part of the cytoplasm of the zygote is derived from the cytoplasm of the $F-$ cell. If that cytoplasm is loaded with streptomycin, that cell is incapable of further development. If there are ways of removing the streptomycin or of inactivating it, there might be further development.

It should be pointed out that the viable counts that are gotten on cultures so-called killed with streptomycin are extremely variable, depending on the details of the conditions of plating, which shows in a way that one has to be very careful in speaking of cells that are "killed" in this particular

province. One should speak quite specifically of which functions are reversibly, and which are irreversibly, inhibited at that time.

I am trying to narrow down what the experiment shows. You can offer one specific hypothesis to explain it; I can offer some more. But in order not to enumerate hypotheses at great length, one can make the generalization that the experiment does indicate that the larger part of the cell substance of the zygote does come from the $F-$ cell. But that is equally compatible with quite a range of hypotheses about what it is that the $F+$ cell contributes, so long as that does not include a lot of cytoplasm containing streptomycin.

PLOUGH: I have a question on a point which both you and Dr. Zinder mentioned. In your recently published studies with Stocker and Zinder on transduction of *Salmonella* antigens, you found that, ordinarily, it was not the specific flagellar antigen of the donor strain which was transduced, but rather the filterable agent (FA) induced the reappearance of the flagellar antigen which the recipient strain presumably had originally. This appears to me to be more easily explanable as a reversion caused by a general mutagenic action of FA.

LEDERBERG: This was ruled out by comparing the effectiveness of phage that had been grown on the nonmotile recipient with phage grown on other motile or nonmotile strains, or to be sure that *strain* homology was not involved, on rare spontaneous motile reversions when these could be obtained. In no case did the phage grown on a given nonmotile indicator confer motility upon it, whereas the other phage preparations were almost always effective. By testing different nonmotile strains against one another, seven distinct groups, presumably different mutant loci, were identified that involve flagellar formation; two which affect their ability to function if formed, and two concerned with their antigenic content. The fact that motile transductions usually gave bacteria that had restored their innate antigenic potentiality simply means that different genetic factors determine whether flagella should be formed

at all, and what their antigenic potentialities would be. This would be, by the way, an almost trivial instance of multigenic control of an antigen, except that we can identify the organelle that underlies the antigen. I did not have the time to go into the dynamics of phase variation; it fits in very nicely with the product story.

HOTCHKISS: I agree with Dr. Lederberg that it is going to be very important to study individual pedigrees in transformed populations. My remarks in this symposium show that we have already started along that line. We have also been using streptomycin resistance to select, immediately within the same hour that DNA was added, cells which have been destined to be changed by the DNA. These were spread on agar and at various times the segregation of the streptomycin resistance and factors linked to it were studied.

As to nomenclature, I think it would be well if we pointed out explicitly that the word "transformation" has disadvantages since it comes from general usage and is adapted for a rather specific sense. But it does have historical value. Many people know what bacterial transformation means. Therefore, I should like to recommend that we retain "transformation" as the generic term, and save "transduction" for the phage-mediated transformations. It seems inadvisable to use the term "transforming principle" except when talking about an abstract principle, rather than an actual material. The term "transforming agent" can be used in reference to a material entity; if it is a phage, it becomes a transducing agent.

LEDERBERG: No one can deny that in all these experiments cells are being transformed, or rather their properties are being altered. In that context, there is no objection at all.

HOTCHKISS: Also, one may have a transformed cell, or "transformant," while you have defined transduction so that it is only the character which is transduced.

LEDERBERG: Precisely. I hope I kept that straight: the transduction of a character from cell A to cell B which results

in the transformation of cell A to type A. I see no reason at all for not using both terms for what they do mean, as the occasion demands. For the same reasons you have indicated, that "transformation" was so vague (meaning, essentially, change) it has been applied to several phenomena with no implication of genetic transfer, even by Griffith himself, in the change from smooth to rough. There is also the transformation of vegetative phage into mature phage, and so on. Perhaps another term is needed to distinguish phage-mediated transductions (or transformations), though perhaps we ought to learn a little more about them first. But "a man coins not a new word without some peril and less fruit; for if it happen to be received, the praise is but moderate; if refused, the scorn is assured" (Ben Jonson).

BERTANI: There seems to be complete similarity of behavior between the transforming agent in your "semiclones" and the phage superinfecting lysogenic cells. Such phage enters the cells (which are carrying a genetically related prophage), but does not affect their ability to grow. As the cells divide, the superinfecting phage does not multiply or multiplies very little, and it is thus diluted out among the growing cells. This state of the superinfecting phage has been called "preprophage." The preprophage, like the transforming agent in the "semiclones," can be considered physiologically active, because, if the cell that carries it lyses, phages of both the prophage and the preprophage types are liberated. The preprophage, also like the transforming agent, has a small chance of "transforming" the cell that carries it, by substituting its own type for the prophage type ("prophage substitution"). When several phage markers are present, this process can be shown to be a true genetic recombination between the prophage and the preprophage.

LEDERBERG: Then the similarity is not complete. Stable transformations are not found out of semiclones. The semiclones occur in clusters from a single parent bacterium.

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